Claims

1. A process for the production of a protein which involves the expression of said protein as a heterologous protein, wherein the correctly folded precursor of the heterologous protein after expression is accumulated in inclusion bodies.
2. A process for the production of a protein which involves performing the biosynthesis of said protein as a heterologous protein in a micro-organism, which process comprises the steps of:

   performing the biosynthesis in a way such that a precursor of the heterologous protein is formed in inclusion bodies of the micro-organism, wherein the precursor is capable of forming the biologically active heterologous protein under non-denaturating conditions;

   isolating said precursor from the inclusion bodies under non-denaturating conditions to thereby form the biologically active heterologous protein.
3. The process for the production of a protein according to claim 1 or 2, wherein the heterologous protein is selected from the group comprising: G-CSF, GM-CSF, M-CSF, EGF, HSA, DNAse, FGF, TNF-alpha, TNF-beta, interferons and interleukins.
4. The process for the production of a protein according to claim 1 or 2, wherein the selected heterologous protein is G-CSF.
5. The process for production of proteins according to any one of the preceding claims, wherein the expression is performed in an organism selected from the group consisting of bacteria and yeasts.
6. The process for the production of a protein according to claim 5, wherein the expression is performed in the bacterium *E. coli.*
7. The process for the production of a protein according to any one of the preceding claims, wherein the heterologous protein is accumulated in the inclusion bodies to a proportion of at least about 10% , preferably at least about 20% and particularly at least about 30%, relative to the total protein mass of the host cell used in the expression system.
8. The process for the production of a protein according to any one of the preceding claims, wherein the inclusion bodies are capable of being mainly dissolved in non-denaturating conditions.
9. The process for the production of a protein according to any one of the preceding claims, wherein the process involves the way of performing the biosynthesis comprising adjusting one or more parameters which are selected from the group

consisting of: temperature of cultivation, composition of the cultivation medium, induction mode, principle of performing the fermentation, addition of an agent capable of causing stress, and co-expression of auxiliary proteins.

10. The process according to claim 9, wherein the temperature of cultivation is between about 20°C and 30°C.
11. The process according to claim 10, wherein the temperature of cultivation is about 25°C.
12. The process according to any one of claims 9 to 11, wherein the adjustment of the induction mode comprises selecting the inducer from the group consisting of IPTG, lactose and NaCl.
13. The process according to claim 12, wherein the selected inducer is IPTG.
14. The process according to claim 13, wherein the concentration of IPTG is in the range from 0.1 mM to 1 mM.
15. The process according to claim 14, wherein the concentration of IPTG is about 0.4 mM.
16. The process according to any one of claims 9 to 15 , wherein the adjustment of the induction mode comprises adding the inducer at the beginning of the fermentation.
17. The process according to any one of claims 9 to 16, wherein the principle of performing the biosynthesis is selected from the group comprising: performing of fermentation in a batch mode, performing of fermentation in a fed batch mode and fermentation in shake flasks.
18. The process according to claim 17, wherein the selected principle of performing the fermentation is a batch mode.
19. The process according to any one of claims 9 to 18, wherein the medium is selected from the group comprising: GYST, GYSP, LYSP, LYST, LBON and GYSPON.
20. The process according to claim 18 wherein the selected medium is GYST or GYSP.
21. The process according to any one of claims 9 to 20, wherein the additive which is capable of causing stress is selected from the group consisting of ethanol and propanol.

22. The process according to any one of the preceding claims, further comprising washing of the inclusion bodies.
23. The process according to claim 22, wherein the washing is performed by using a solution which is selected from the group consisting of Tris/HCl buffer, phosphate buffer, acetate buffer, citrate buffer and water.
24. The process according to claim 23, wherein the concentration of the selected buffer is in the range from about 1 mM to 10 mM.
25. The process according to claim 23, wherein the selected solution is water.
26. The process for production of a protein according to any one of the preceding claims, wherein further comprising solubilisation of the inclusion bodies.
27. A process for the production of a protein using a micro-organism, wherein said protein is expressed as a heterologous protein and is formed in inclusion bodies in said micro-organism, which process comprises the steps of:
isolating said inclusion bodies;
optionally washing said inclusion bodies;
and subjecting said inclusion bodies to a solubilisation treatment under non-denaturating conditions.
28. The process acording to claim 27, wherein the process for the formation of the heterologous protein in inlcusion bodies is performed in accordance with any one of claims 1 to 25.
29. The process according to claim 26 or 27, wherein the solubilisation is performed by using an agent for solubilisation being selected from the group consisting of:urea in non-denaturating concentrations (1–2 M), N-lauroyl sarcosine in non-denaturating concentrations (0.05–0.25% (m/v)), Zwittergents in low, non-denaturating concentrations, non-detergent sulfobetains (NDSBs), betain, sarcosine, carbamoyl sarcosine, taurine, DMSO and a buffer in a high, solubilising concentration, wherein the buffer is selected from the group consisting of: HEPES, HEPPS, MES, ACES, and MES.
30. The process according to claim 29, wherein the selected solvent is N-lauroyl-sarcosine.
31. The process according to claim 30, wherein the concentration of N-lauroyl sarcosine is in the range from about 0.1% to 0.25%.
32. The process according to claim 30, wherein the concentration of N-lauroyl sarcosine is about 0.2%.

33. The process for production of biologically active G-CSF by biosynthesis, wherein parameters for the way of performing the biosynthesis are selected as follows:

- cultivation temperature: about 20-30°C, preferably about 25°C
- composition of cultivation medium: selected from group consisting of GYST, GYSP, LYSP, LYST, LBON and GYSPON, preferably GYST and GYSP
- type of fermentation: batch mode
- induction mode: adding IPTG, in case of an optional preculturing step after said preculturing step, at the beginning of the fermentation, to adjust a final IPTG concentration in the range of about 0.3 to 0.6 mM, preferably to about 0.4 mM.

34. The process for production of biologically active G-CSF according to claim 33, wherein the process further comprises isolating inclusion bodies and washing the isolated inclusion bodies with water.

35. The process for production of biologically active G-CSF according to claim 33 or 34, wherein the process further comprises the solubilisation of inclusion bodies with N-lauroyl sarcosine with the concentration of about 0.1 to 0.25%, preferably about 0.2%.

36. The process according to any one of the preceding claims, wherein the produced biologically active heterologous protein, for example G-CSF, is finally purified by a purification process which is performed under non-denaturating conditions.

37. A use of the biologically active heterologous protein, for example G-CSF, as produced according to any one of claims 1 to 35, and preferably as purified according to claim 36, for the preparation of a medicament.